

April 18, 2002

Laurie Miller  
American Chemistry Council  
Acetic Acid and Salts Panel  
1300 Wilson Boulevard  
Arlington, VA 22209

Dear Ms. Miller:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Acetic Acid and Salts Category, posted on the ChemRTK HPV Challenge Program Web site on October 2, 2001. I commend The American Chemistry Council Acetic Acid and Salts Panel for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its HPV Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA believes that grouping of all 13 chemicals into one category is not supported based on their chemical structural, functional, and toxicological properties. Instead of grouping together all the chemicals into a single category, EPA recommends that the submitter needs to provide separate submissions for each of the separate categories as suggested in the Comments.

EPA will post this letter and the attached Comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The American Chemistry Council Acetic Acid and Salts Panel advise the Agency, within 90 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the HPV Challenge Program Web site "Submit Technical Questions" button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at [tsca-hotline@epa.gov](mailto:tsca-hotline@epa.gov).

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/s/

Oscar Hernandez, Director  
Risk Assessment Division

Attachment

cc: W. Sanders  
A. Abramson  
C. Auer  
M. E. Weber

**EPA Comments on Chemical RTK HPV Challenge Submission:  
Acetic Acid and Salts**

**SUMMARY OF EPA COMMENTS**

The sponsor, the American Chemistry Council Acetic Acid and Salts Panel, submitted a test plan and robust summaries to the EPA for the Acetic Acid and Salts category dated July 5, 2001. EPA posted the submission on the ChemRTK HPV Challenge Web site on October 2, 2001. The Acetic Acid and Salts category includes 13 substances (see Category Definition section below).

EPA has reviewed this submission and reached the following conclusions:

1. Category Justification. EPA believes that grouping of all 13 chemicals into one category is inappropriate based on their chemical structural, functional, and toxicological properties. From a structural perspective, the proposed category may be directed as follows: 1) citric acid and salts; 2) malic acid; 3) fumaric acid; 4) acetic acid and salts (except manganese); and 5) acetic acid, manganese salt. The latter is isolated from the acetic acid and salts group based on the toxicity of manganese. In addition, suitable arguments need to be developed to associate malic and fumaric acids with one of the dominant groups (acetic and citric acids). EPA recommends that the submitter needs to provide separate submissions for each of these categories instead of grouping together all the chemicals into a single category.
2. Physicochemical and Environmental Fate Data. The submitter estimated the transport and distribution of these chemicals using a Level I Model. EPA recommends using the EQC Level III model (See Specific Comments on the Robust Summary).
3. Health Endpoints. Grouping of all chemicals into a single category and extrapolating data between citric acid and other members of the category is inappropriate based on the chelating properties of citric acid that are toxicologically significant. In addition, the submitter needs to provide a discussion on the potential toxicity of manganese and the toxicity that may result from the chelation of cations.

Overall, the summaries provided for health effects studies lack data necessary for an independent assessment of their quality. The submitter needs to provide revised robust summaries viewing the original publication or study report to obtain the necessary information.

4. Ecotoxicity. The robust summaries for acute toxicity did not provide sufficient details to fully evaluate the associated studies. EPA reserves judgement on the adequacy of the studies for invertebrates and algae pending submission of the missing critical information. Fish acute toxicity data appear adequate, however, the submitter needs to enhance the robust summary for this endpoint as well.

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

**EPA COMMENTS ON THE ACETIC ACID AND SALTS CATEGORY  
CHALLENGE SUBMISSION**

**Category Definition**

The submitter defines the acetic acid and salts category as a set of structurally related compounds containing one or more carboxylic acids. In addition to acetic acid and its salts, the category also includes fumaric, malic, and citric acids and selected salts of citric acid. These chemicals occur naturally as constituents in plants and animals, play an important role in tricarboxylic acid cycle and have a commercial use as food additives. The category contains 13 substances: acetic acid (64-19-7); acetic acid, ammonium salt (631-61-8); acetic acid, potassium salt (127-08-2); acetic acid, sodium salt (127-09-3);

acetic acid, calcium salt (62-54-4); acetic acid, magnesium salt (142-72-3); acetic acid, manganese salt (638-38-0); fumaric acid (110-17-8); malic acid (6915-15-7); citric acid (77-92-9); citric acid, sodium salt (unspecified number of sodium atoms)(994-36-5); citric acid, tripotassium salt (866-84-2); and citric acid, trisodium salt (68-04-2).

### Category Justification

The submitter states that the 13 substances in the proposed category were grouped by "...their close structural relationships, their natural occurrence in plants and animals, and their fundamental role in cell metabolism." The submitter also states that "[t]he toxicity of each compound is driven by acetate, with the cations playing a minor role." None of these points, however, are explained in detail in the test plan.

While the submitter's reference to the "close structural relationship" of the chemicals in this category clearly refers to the organic portion of each member (e.g., acetate), category members include mono-, di-, and tricarboxylic acids with either saturated or unsaturated chains. The only obvious structural feature that these compounds share is their carboxylic acid function.

Indeed, the carboxylic acids in the category are present naturally and are components of the tricarboxylic acid cycle (an important energy production pathway based in the mitochondria). This cycle, however, uses only acetate to produce two CO<sub>2</sub> molecules and energy, but both generates and consumes citrate, fumarate, and malate during that process. Therefore, the presence of citrate, fumarate, and malate in this cycle cannot be used to describe their metabolism by likely exposure routes. In addition, the role of acetate in the tricarboxylic acid cycle cannot be used to completely describe its metabolism, because acetate in the cycle is normally generated from fatty and amino acids and must be present as acetylCoA. Added acetate would not necessarily be metabolized by an organism using this cycle. Finally, the tricarboxylic acid cycle pertains only to mitochondrial acetate, and may pertain only to this compartment of the cell. Using this reasoning to describe the metabolism of the category members, therefore, is not supported by the description presented in the test plan, and the submitter needs to provide additional information.

The submitter uses the data supplied in the robust summaries and the assumption that the organic portion of these compounds is the primary toxic agent to construct a matrix of available data and "read-across patterns." The submitter concludes that all HPV Challenge Program endpoints are satisfied and that additional testing is not required. An integral part of this Test Plan is the premise of the submitter that (1) the salts "readily dissociate in solution," and (2) that "the cationic portion of the salt (e.g., Ca<sup>2+</sup>, Mg<sup>2+</sup>, , Mn<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>) does not significantly affect the relative toxicity of these compounds." While the first premise appears true for the salts in this category, this does not mean that these acids and cations behave identically. This is particularly true of citric acid, which has the potential to chelate cations such as Mg<sup>2+</sup>, Ca<sup>2+</sup>, and iron. Since chelation of these ions may be toxicologically important, it may be inappropriate either to extrapolate data from other members of the category to citric acid or to extrapolate citric acid data to the other acids and salts. The second premise, that the cationic portions of these salts are toxicologically insignificant, was not supported by an adequate discussion in the submission. For example, manganese ion is toxic to fish and daphnids. In addition, a repeated-dose toxicity study on acetic acid, manganese salt showed neurotoxic signs in mice and a weak positive response in bacterial gene mutation test.

Based on the above discussion, EPA believes that grouping of all 13 chemicals into one category is inappropriate. The chemicals in this category would be addressed better if they are separated into the following five categories: 1) citric acid and salts; 2) malic acid; 3) fumaric acid; 4) acetic acid and salts (except manganese); and 5) acetic acid, manganese salt. The latter is isolated from the acetic group based on the toxicity of manganese. Suitable arguments need to be developed to associate malic and fumaric acids with one of the dominant groups (acetic and citric acids).

## Test Plan

### Physicochemical Properties (melting point, boiling point, vapor pressure, water solubility, and partition coefficient)

The submitter needs to provide octanol/water partition coefficient values for acetic acid, magnesium salt; acetic acid, manganese salt; and citric acid, sodium salt.

### Environmental Fate (Photodegradation, Stability in Water, Biodegradation, Fugacity)

*Chemical Transport and Distribution in the Environment.* The submitter indicates in its Test Plan that it estimated the transport and fugacity of these chemicals using the level I Fugacity Model. EPA recommends using the EQC Level III model (see Specific Comments on the Robust Summaries).

### Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

Sufficient data may be available for most of these chemicals to address endpoints for the HPV Challenge Program. The health effects information submitted for these chemicals consisted of abbreviated summaries. All of the summaries were inadequate for the purposes of the HPV Challenge Program. The submitter needs to review the original references for all studies, and revise the summaries to include the information on study design, experimental methods, and results to permit an independent assessment of their adequacies.

*Acute Toxicity.* Data are available to characterize the acute oral toxicity of compounds in the category. Although none of the summaries themselves were adequate, LD<sub>50</sub> values for all chemicals tested in mice and rats support the submitter's conclusion that additional testing for this endpoint is not required.

*Repeated Dose Toxicity.* Summaries were submitted for acetic acid and its sodium and manganese salts, fumaric acid, malic acid, and citric acid and its sodium salt. None of the submitted studies were designed or conducted to specifically evaluate the toxicity of these compounds and all appear to have significant limitations. It is inappropriate to extrapolate citric acid data to other chemicals because of its chelating properties. In addition, manganese is a potential neurotoxicant and there is potential for accumulation of this metal in the brain (study by Komura and Sakamoto (1992)), suggesting that the manganese salt is not an appropriate analog for other members of the category. If the primary sources are reviewed and additional information is provided on study design and results, EPA believes that the data may be adequate to address this endpoint for all five EPA proposed categories.

*Genotoxicity.* The existing data for bacterial gene mutation are adequate to characterize the endpoint for the purposes of the HPV program for acetic acid and its sodium salts, fumaric acid, and citric acid and its sodium salt. The summaries had deficiencies in reporting of experimental detail. A similarly flawed negative study summary on malic acid was not supported by any additional studies; the submitter needs to provide a more complete description of this study before its adequacy can be assessed. The submitter needs to provide bacterial gene mutation data (in vitro and in vivo) on acetic acid, manganese salt.

*Reproductive Toxicity.* The existing data for reproductive toxicity are not considered adequate to characterize the endpoint for the proposed categories. The study for citric acid may be adequate after review of the primary study; however, the adverse effects observed in this study were attributed to chelation of essential elements. Since the chelation properties of citric acid differ from those of the other compounds in the category, citric acid is not an appropriate analog for characterization of the reproductive toxicity of the other members of the category. The submitter needs to include additional information on the studies provided and a more detailed discussion of the reproductive toxicity of the cations of these acids. The

study on fumaric acid was inadequate because it was a single exposure level dietary study in which only 2 female guinea pigs were exposed to the test substance with no adverse effects were reported at the dose level that was below that of a limit dose.

*Developmental Toxicity.* The study on acetic acid had insufficient details on experimental design and results to permit evaluation of the adequacy of the data, while the study of the sodium salt of this acid was inadequate because a short exposure period was used (gestation days 8 through 12). Data for malic and citric acid were considered inadequate to characterize the endpoint because they were based on testing of single doses well below the criterion for an OECD limit dose. No data were available for fumaric acid. The submitter's conclusion that adequate data exist for this endpoint is not supported by the data submitted, and either discussion with adequately summarized studies or additional testing is needed for developmental toxicity. The observation that the chelating properties of citric acid may contribute to its toxicity, suggests that other (non-chelating) compounds in the category are not appropriate analogs for citric acid and its salts. In addition, the manganese salt of acetic acid showed developmental effects in birds in a non-conventional fertile single-comb chicken eggs study, showing manganese plays a role in early development. (The submitter needs to search ATSDR'S TOX PROFILE for manganese reproductive and developmental toxicity.)

#### Ecotoxicity (fish, daphnid and algal toxicity)

Deficiencies in the robust summaries prevented an independent determination of data adequacy for the existing studies. The submitter needs to provide sufficient information before the data can be evaluated (see Specific Comments on Robust Summaries). Although there were deficiencies in the study summary, EPA reviewed the in-house study report for fish acute toxicity of acetic acid (EPA-600/3-76-097) and concluded that this endpoint has been addressed.

### **Specific Comments on the Robust Summaries**

#### Physicochemical Properties

On page 7 of the Robust Summary, the submitter reports an estimated vapor pressure of 19.6 hPa (14.7 mm Hg) at 25 °C for acetic acid, calcium salt. This value is much higher than that would be expected for a salt and is probably not accurate. The submitter's estimates for the remaining salts in this category are reasonable since salts generally have vapor pressures of less than  $1 \times 10^{-6}$  mm Hg at 25 °C.

#### Environmental Fate

The submitter estimated the transport and distribution of these chemicals using a Level I Model. EPA recommends using the EQC Level III model, which is more realistic and useful for estimating a chemical's fate in the environment. In order to develop the Level III fugacity model, EPA recommends using the EQC Level III model from the Canadian Environment Modeling Centre at Trent University, which allows full control of data inputs. This model can be found at the following web address:  
<http://www.trentu.ca/academic/aminss/envmodel>.

#### Health Effects

Out of 82 robust summaries submitted for this category, 24 were not directly relevant to the evaluation of SIDS/HPV endpoints, on the basis of the route of administration or the use of non-mammalian tests. None of the summarized studies were GLP- or OECD-compliant and all summaries lacked information necessary for a complete evaluation of study reliability and adequacy. In many cases, the summaries were based on secondary sources of information, which did not provide a sufficient level of detail for the purposes of the HPV Program. The submitter needs to review the primary study reports or publications and revise the summaries to include details of study design, methods, and results.

*Acute Toxicity.* None of the twelve summaries were GLP- or OECD-compliant and all summarized studies had one or more deficiencies in experimental design, conduct, or reporting when compared to OECD guidelines. The submitter needs to revise the summaries to include relevant experimental details.

*Repeated Dose Toxicity.* Twelve oral summaries and one inhalation repeated dose summary were submitted. The summaries were deficient in reporting of experimental details. One study on citric acid was considered adequate to characterize the repeated-dose toxicity of this compound. One study each for acetic acid, sodium salt, fumaric acid, and malic acid may be adequate to characterize the endpoint, provided the summaries are revised to include missing information on study design, methods, and reporting of results. The adequacy of several summarized studies was limited by the use of a single dose below the limit dose criterion and/or by lack of information on control response.

*Genetic Toxicity.* All summaries lacked information on study design, methodology, and results. The experimental endpoint for summary 5.5.A.(i) could not be determined from the summary. It was not clear whether the zinc salt of acetic acid was tested in the bacterial mutagenicity assay described in summary 5.5.A.(c).

*Reproductive Toxicity.* Four summaries were submitted on the reproductive toxicity of fumaric acid, citric acid, or citric acid, sodium salt. The submitter needs to provide revised summaries and a more detailed discussion of the reproductive toxicity of the cations of these acids.

*Developmental Toxicity.* Five out of eleven study summaries submitted for the developmental toxicity endpoint were not reviewed because they described assays in chicken eggs or *Drosophila*, which are not considered suitable for SIDS/HPV purposes. Developmental studies on acetic acid may be adequate to characterize this endpoint, provided the summaries are revised to include missing experimental details, such as the number of animals tested per dose, endpoints evaluated for fetuses for malformations and variations, maternal and developmental endpoints, and statistical methods used to analyze data. Studies on acetic acid sodium salt, malic acid, and citric acid are not considered adequate for characterizing developmental toxicity, because of inadequate exposure duration (only for days 8 through 12 of gestation) or the use of doses below the limit dose criterion in the absence of demonstrated maternal or fetal toxicity.

### **Ecotoxicity**

The robust summaries for acute toxicity endpoints did not provide sufficient details to fully evaluate the associated studies. Most of the robust summaries lack critical data elements. The submitter needs to provide the following missing information for all robust summaries including the EPA fish toxicity study: test substance purity, use of analytical monitoring, number of organisms per test, temperature, DO, pH, water hardness, number of replicates, and type of test.

*Aquatic Plants.* The submitter needs to enhance the robust summaries for fumaric (72 hr) and citric acid (96 hr) studies. The other studies are inadequate because they were conducted for nonstandard endpoints and do not need revision.

### **Followup Activity**

EPA requests that the Submitter advise the Agency within 90 days of any modifications to its submission.